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White Oaks Genetic and Chemical Diversity Affect the Community Structure of Canopy Insects Belonging to Two Trophic Levels

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Abstract: The hybridization phenomenon increases genetic diversity and modifies recombinant individuals' secondary metabolite (SMs) content, affecting the canopy-dependent community. Hybridization events occur when *Quercus rugosa* and *Q. glabrescens* oaks converge in sympatry. Here, we analyzed the effect of the genetic diversity (*He*) and SMs of *Q. rugosa*, *Q. glabrescens* and hybrids on the community of gall-inducing wasps (Cynipidae) and their parasitoids on 100 oak canopy trees in two allopatric and two hybrid zones. Eighteen gall wasp species belonging to six genera and six parasitoid genera contained in four families were identified. The most representative parasitoid genera belonged to the Chalcidoidea family. Abundance, infestation levels and richness of gall wasps and their parasitoids registered the next pattern: *Q. rugosa* higher than the hybrids, and the hybrids equal to *Q. glabrescens*. Oak host genetic diversity was the variable with the highest influence on the quantitative SMs expression, richness and abundance of gall wasps and their parasitoids. The influence of SMs on gall wasps and their parasitoids showed the next pattern: scopoletin > quercitrin > rutin = caffeic acid = quercetin glucoside. Our findings indicate that genetic diversity may be a key factor influencing the dynamics of tri-trophic interactions that involve oaks.

Keywords: secondary metabolites; hybridization; white oaks; tri-trophic interactions

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1. Introduction

For decades, we have known that variation in plant populations' structure and genetic diversity leads to changes in their associated arthropod communities. Herbivorous arthropods can distinguish between different plant species [1] and their hybrids [2], as well as between different plant genotypes within a single species [3]. Also, genetic variation in plants affects herbivores and can extend to higher trophic levels among different

plant species [4], among putative plant species and their hybrids [5] and among plant genotypes [6]. Consequently, this discrimination between plants can influence arthropod diversity and community structure [7]. Also, some studies have shown that increasing genetic diversity within interbreeding plant systems (e.g., hybrids and single plant species) increases arthropod diversity and community structure [8,9]. Plant genetics play an important role in SMs production and plant architecture [10–13]. These differences impact the availability, quality and quantity of resources for herbivores, influencing their performance, population density, community structure and evolutionary patterns [14–16]. Furthermore, the effects of plant variation on herbivorous arthropods extends to higher trophic levels, resulting in changes in the composition, abundance and diversity of predators and parasitoids among different plant species and between parental plant species and their hybrids [17]. However, rarely have chemical traits in plant species been correlated with plant genetics and arthropod community structure [18,19].

Oaks (Fagaceae: *Quercus*) are considered the most abundant woody plant group in the Northern Hemisphere [20], with an estimated 500 species worldwide [21]. Mexico is home to 30.3% of oak species and is considered one of the most important diversification centers of the genus [22]. Oaks are important for the ecological functions they perform (e.g., soil fertilization, nutrient cycling and water balance) [23]. Oaks dominate the canopy of forest ecosystems and have an extensive and complex network of interactions with other organisms, such as epiphytic plants, mammals, birds, fungi and arthropods [24]. In particular, oak distribution, abundance and species richness have significantly influenced the distribution and species richness of oak gall wasps [8].

One of the characteristics of the genus *Quercus* is the high frequency of hybridization and introgression events between species belonging to the same section [25]. Hybridization is a form of genetic exchange, and several studies on oaks have documented that the increased genetic diversity of the species involved [26,27] promotes variation in morphological, chemical and phenological characteristics, among others [28–30], with important consequences at the community structure level [31,32]. For example, arthropods respond to certain characteristics of their host plant species, such as biomass, foliar nutritional quality and SMs, attributes that have a genetic basis [33]. These characteristics may influence the oviposition and feeding preferences of the arthropods associated with oaks and, consequently, their distribution and abundance [34].

Secondary metabolites are chemical compounds derived from the primary metabolism but not related to the normal development and/or growth of plants [35]. The hybridization process modifies the metabolic pathways of interacting species, creating differences in SMs expression (type, concentration and quality) [36]. Secondary metabolite expression in hybrid plants can vary qualitatively and quantitatively [37]. Qualitatively, hybrids can express all or some of the SMs found in parental taxa or even new SMs. Quantitatively, the concentration of SMs can be higher, intermediate, lower or similar to the concentration in one or both parental taxa [38,39]. These compounds are associated with plant survival and adaptability [40], and in most cases, SMs are involved in defense against herbivory [41].

Studies of the genus *Quercus* have reported that hybridization induces variation in the qualitative and quantitative expression of SMs [42,43]. In general, the most frequently reported SMs in oak leaves are flavonoids, terpenoids, tannins and aliphatic compounds [43,44], which can act as insect attractants, feeding inhibitors, regulators of the respiratory chain (terpenoids–tannins), cytotoxins and regulators of larval development (flavonoids). These compounds are associated with generalist and some specialist herbivores [45].

The endophagous, gall-inducing wasps belonging to the Cynipidae family (Hymenoptera: Cynipidae: Cynipini Tribe) associated with the genus *Quercus* are considered one of the most specialized groups of herbivorous insects [46]. It has been proposed that

cynipids can detect small physiological, chemical or phenological changes in their host oaks, which allow them to "select" oviposition and/or feeding sites [47,48]. The galls induced by cynipids form a tri-trophic system with great ecological activity [49], harboring a closely associated community of guest organisms and parasitoids, particularly from the Chalcidoidea family [48]. The parasitoids associated with cynipids are specific to them; it has been reported that their communities may be affected by the genetic attributes or chemical defense mechanisms of plants [9,50]. Therefore, oaks and their associated gall insects and parasitoids represent an excellent system to study the effect of the genetic and chemical variations that result from hybridization events on cynipid communities and their associated parasitoids.

The identification of hybrid individuals is crucial in studies related to hybridization. Hence, different markers have traditionally been employed, emphasizing genetic and chemical markers (SMs) [43,51–53]. Given that DNA-based markers depend on genotypic data, they are viewed as the most effective markers for identifying hybrids, highlighting Simple Sequence Repeats (SSRs; [33]), which can effectively differentiate the heterozygous condition and are clearly favored over dominant markers. Regarding SMs and due to their oligogenic control, they have a much more predictable inheritance compared to morphological traits, which are generally influenced by polygenic control and the emergence of transgressive characters [54]. Consequently, several researchers have utilized SMs alongside genetic markers to detect hybrid individuals within natural populations. Finally, hybrids' SMs play a crucial role, possibly contributing to the success of hybrid populations by expressing unique combinations of SMs that enhance their defense against herbivores [54].

A previous study reported genetic (microsatellite) and chemical (flavonoid) evidence of hybridization in a white oak complex formed by *Q. glabrescens* and *Q. rugosa* [43]. The authors detected specific chemical markers for each parental species and a complementary pattern of the qualitative expression in hybrids, that is, the presence of flavonoids from the parental species.

The aim of this study was to evaluate the effect of the genetic diversity and SMs of a *Q. glabrescens* × *Q. rugosa* complex on their associated community structure of gall-inducing wasps and parasitoids. The following questions were posed: (1) Do hybridization events between *Q. glabrescens* and *Q. rugosa* promote quantitative differences in the pattern of flavonoid and coumarin expression in their hybrids? (2) Is there a relationship between the genetic diversity and SMs variation in the *Q. glabrescens* × *Q. rugosa* complex and the associated community of gall-inducing insects (Cynipidae) (3) What is the level of influence of genetic diversity and SMs on the community of gall-inducing wasps? (4) Do the genetic and chemical attributes of the host oak affect the community of parasitoids associated with gall-inducing wasps?

2. Materials and Methods

2.1. Study Species

Quercus glabrescens Benth. and *Q. rugosa* Née are abundant species in the four study sites. *Q. glabrescens* includes large trees of up to 20 m in height, with a trunk diameter of 1 m. *Q. rugosa* includes large trees of up to 25 m in height, with a trunk diameter of 1 m. Both species show a wide geographical distribution in Mexican mountains and canopy dominance [43]. In a previous study, Castillo-Mendoza and collaborators [43] documented hybridization between both *Quercus* species in sympatric zones.

2.2. Study Sites and Population Sampling

The oak trees sampled in the present study correspond to the populations previously identified morphologically and genetically by Castillo-Mendoza et al. [43]. Specifically, we used the two sympatric sites between *Q. glabrescens* and *Q. rugosa* and the allopatric popula-

tions of these species. In total, 100 individuals belonging to four populations (two allopatric [20 trees/site, one for each parental species] and two sympatric sites [30 trees/site]) were analyzed (Table 1). We selected this sample size considering that previous studies have used similar sample sizes and found significant and robust results [8,9,18]. To minimize the influence of environmental and spatial variables on gall-inducing wasps and their parasitoid communities, all study sites shared the following characteristics: geological history (all localities belonged to the central part of Mexico, originating during the Pliocene–Quaternary [55]), weather (sub-humid temperate [56]), altitude (between 2318 and 2667 m), vegetation type (mature oak) and soil type (volcanic origin: histosol [57]). Also, all sampled individuals were mature trees without apparent damage by other herbivores.

Table 1. Locality name, state, altitude, geographic coordinates and sample size for the collecting sites of *Q. glabrescens* × *Q. rugosa* in Central Mexico.

Location	N	State	Altitude (m)	Coordinates (N–W)	Taxa
Allopatric stand					
Tlaxco	20	Tlaxcala	2588	19°41′44.7″–98°4′49.1″	*Q. glabrescens*
Coajomulco	20	Morelos	2667	19°2′3.5″–99°11′54.1″	*Q. rugosa*
Sympatric stand					
Huitzilac	30	Morelos	2318	19°1′57″–99°16′34″	*Q. glabrescens*, *Q. rugosa*, hybrid
Omitlán de Juárez	30	Hidalgo	2522	20°9′57″–98°39′16″	*Q. glabrescens*, *Q. rugosa*, hybrid

2.3. Molecular Data

In a previous study, we found that molecular markers (microsatellites, SSRs) supported the hybridization hypothesis between *Q. rugosa* and *Q. glabrescens* [43]. The genetic diversity analyses were performed with eight nuclear SSR primers: ssrQpZAG110 [58], ssrQpZAG11, ssrQrZag56 [59], Quru-GA-0A01, Quru-GA0E09, Quru-GA-0C11, Quru-GA-1C08 and Quru-GA-1F07 [60]. All these nSSRs showed polymorphisms among the individual trees of the *Q. glabrescens* × *Q. rugosa* complex. For more technical details, see Castillo-Mendoza et al. [43].

2.4. Chemical Data

In an earlier study, we identified nine flavonoids and one coumarin of the *Q. glabrescens* × *Q. rugosa* complex [43]. In total, 48 individuals [*Q. glabrescens* (n = 18), *Q. rugosa* (n = 18), hybrids (n = 12)] were quantitatively analyzed using the same extracts obtained by Castillo-Mendoza et al. [43]. Column chromatography was employed to purify the extracts, and we obtained 10 mg of pure compound per population. Due to their high heritability and specificity, flavonoids have been the most studied compounds [13] and are used as chemical markers to diagnose plant hybridization [30].

HPLC was employed to determine flavonoid concentrations through calibration curves, which are generated by known commercial standards for each compound (the caffeic acid, kaempferol glucoside, quercetin, quercitrin, rutin and scopoletin present in *Q. rugosa*, hybrids, and *Q. glabrescens* ([see Appendix A] [Sigma-Aldrich Chemical Co., St. Louis, MO, EUA]). Chemical separation was carried out in a reversed-phase column supelcosil (rp-18, 25 cm, 4 um) under a gradient TFA/acetonitrile (flow = 0–9 mil/min; vol. 10 µL, wavelength 350 nm). Afterward, the area of each known standard was determined, and a graph correlating to the area of the peak, with its mass, was created. Finally, the SMs concentration was calculated as the mean ± standard error (µg/mL^{-1}) on a dry weight (DW) basis.

By measuring the peak areas, the calibration curves were linear (r^2 > 0.998), in the concentration range of 12.5 to 200 ng. Six control standards containing 12.5, 25, 50, 100

and 200 $\mu g/mL^{-1}$ in triplicate were used (10 µL injection each) to ensure accuracy and precision, where RSD (%) values were within 2% of the actual concentrations (Table 2). This confirmed the precision and validation of the method. The method has the advantage of using a simple gradient elution in the reverse phase without adding buffers.

Table 2. Regression analysis, limits of detection and limits of quantification for the six analytes of the assay.

Secondary Metabolite	Detection Limits (nm)	Regression Equation	r^2	Linear Range ($\mu g/mL^{-1}$)	LOD ($\mu g/mL^{-1}$)	LOQ ($\mu g/mL^{-1}$)
quercetin-3-O-rutinoside (rutin)	312	$Y = 17{,}931\ X - 99{,}324$	0.9996	12.5–200	12.33	37.38
quercetin-3-O-glucoside (quercetin)	312	$Y = 22{,}019\ X - 219{,}819$	0.9955	12.5–200	10.78	32.67
caffeic acid	312	$Y = 24{,}759\ X - 91{,}894$	0.9991	12.5–200	4.10	12.45
scopoletin	330	$Y = 25{,}131\ X + 66{,}600$	0.9991	12.5–200	8.85	17.74
kaempferol-3-O-glucoside (kaempferol glucoside)	312	$Y = 11{,}149\ X + 17{,}786$	0.9980	12.5–200	7.91	23.99
quercetin-3-O-rhamnoside (quercitrin)	312	$Y = 7837\ X + 4622$	0.9996	12.5–200	4.49	13.61

Y = the peak area in UV chromatograms monitored at 312 nm, X = compound concentration injected, r^2 = determination coefficient, LOD = limits of detection, LOQ = limits of quantification.

For this study, we identified two new metabolites using the same chemical methodology and individuals employed previously by Castillo-Mendoza et al. [43] (Table 2; Appendix A). Also, we determined the concentration of four SMs [caffeic acid, quercetin-3-O-glucoside (=quercetin), quercetin-3-O-rutinoside (=rutin), quercetin-3-O-rhamnoside (=quercitrin)] identified previously by Castillo-Mendoza et al. [43] and two new SMs characterized in the present study [(kaempferol-3-O-glucoside (=kaempferol glucoside) and scopoletin]. Finally, flavonols 1-5 and the alkyl coumarate were not quantitatively analyzed, because it was not possible to determine their specific identification (see Table 2 in Castillo-Mendoza et al. [43]). From now, we will use the simple nomenclature for each compound (Appendix A).

Also, for statistical analysis, the values below the detection limit of the HPLC were assigned as half of this limit [61].

2.5. Canopy Gall-Inducing Wasp Communities and Associated Parasitoids

The gall-inducing wasp and parasitoid community structure associated with *Q. rugosa* (n = 40) and *Q. glabrescens* (n = 40), including their hybrids (n = 20), was analyzed in the same 100 individuals as in Castillo-Mendoza et al. [43]. We selected oak individuals between 10.0 and 13.4 m (mean ± standard error) (*Q. rugosa*: 10.59 ± 0.11, *Q. glabrescens*: 11.58 ± 0.13, hybrid: 10.89 ± 0.09) in height and with 10.2–13.2 m^2 (*Q. rugosa*: 14.95 ± 0.35, *Q. glabrescens*: 13.02 ± 0.37, hybrid: 11.83 ± 0.65) of crown cover. Oak gall-inducing wasps and parasitoid were sampled in April and December 2016. The infestation by gall-inducing wasps associated with each host tree was estimated using four randomly selected branches and 200 leaves (50 leaves per branch) in the middle part of the canopy. For each insect species, an average infestation value was estimated (number of galls/200 leaves × 100) over the four branches. Galls collected in each host tree were separated into the morphospecies level, placed in previously vouchered plastic containers and transported to the laboratory where the insects emerged. Wasps were identified to the finest possible taxonomic level [62–74], and their parasitoids were identified at the genus level [75–78].

2.6. Statistical Analysis

2.6.1. Genetic Diversity of Host Plant

To estimate the influence of the *Q. rugosa*, *Q. glabrescens* and hybrids' genetic diversity on SMs, canopy gall-inducing insects and their parasitoids, the expected heterozygosis (*He*: the probability that two alleles taken at random from the population are different) was

used to analyze the genetic diversity at the population level. The genetic analyses were performed with the same eight nSSR primers reported by Castillo-Mendoza et al. [43]. *He* was used because it is frequently employed to evaluate the influence of population genetic diversity on the community structure [8,9,79]. The software used for the mean expected heterozygosity (*He*) was Popgene v. 1.31 [80]. Thereafter, a Kruskal–Wallis analysis of variance was conducted to determine significant differences in genetic diversity values among populations [81].

2.6.2. Community Structure of Canopy Gall-Inducing Insects and Associated Parasitoids

An analysis of variance (ANOVA) was conducted ([Model III (orthogonal)] [81]) to determine the effect of oak host taxa (*Q. rugosa*, *Q. glabrescens*, and hybrid: independent variable) on species richness, abundance and infestation percentage of gall-inducing insects and their associated parasitoids (dependent variables). Also, the Shannon–Wiener diversity index (*H′*) was estimated to characterize species diversity in a community; subsequently, the index (*H′*) was compared between pairs of taxa with a randomization test (delta, δ); this test re-samples 10,000 times from a distribution of species abundances produced by a summation of the two samples [82]. In order to satisfy parametric test assumptions, percentage data were corrected as X = arcsin (%)½, and discontinuous data were transformed as X = (X) ½ + 0.5 [81]. A Tukey test was carried out to determine differences in mean infestation (%), abundance and species richness between hybrid oaks and parental species [81]. The software used for statistical analysis was STATISTICA 8.0 [83] and Species Diversity and Richness version 3.03 [84].

2.7. Influence of Host Taxa, Genetic Diversity and Secondary Metabolites on Canopy Gall-Inducing Insects and Associated Parasitoids

We used a multiple regression approach to examine whether the host taxa (*Q. rugosa*, *Q. glabrescens* and hybrid) genetic diversity levels (*He*) and SMs (caffeic acid, quercetin glucoside, rutin, quercitrin, scopoletin and kaempferol glucoside) influence the canopy gall-inducing wasps and associated parasitoids. This analysis was helpful to determine the relative contribution from each factor on the abundance and species richness variation of gall-inducing wasps and their parasitoids. We used a standard least squares model with a partial (type III sums of squares) error structure and genetic diversity and SMs as our factors. We excluded variables with a non-significant correlation coefficient ($p > 0.05$) to improve the analysis. Considering that *He* is a variable that contributed to SMs expression, we were interested in determining whether the host *He* can predict the SMs. Therefore, we used simple linear regressions.

The Shannon-Wiener diversity index (*H′*) vs. species richness (*S*) ($r = 0.830$, $p < 0.01$), galls abundance vs. galls infestation levels ($r = 0.957$, $p < 0.001$) and parasitoids abundance vs. parasitoids infestation levels ($r = 0.597$, $p < 0.001$) variables were correlated. To assess the relationship between *He* and SMs, regression analyses were conducted only with *S* and the abundance of gall-inducing insects and their parasitoids variables. The software used for statistical analysis was STATISTICA 8.0 [83].

We also built networks comprising pathways that facilitate the measurement of the negative and positive influence of genetic diversity and SMs on the richness and abundance of the gall inductor wasps and their parasitoids. We used the linear regression method to quantify the causal relationship between dependent and independent variables. Specifically, we used the slope value of the interpolated straight line derived from the linear regression method to quantify the relationship trend either in an upward or a downward direction. We used values between 0° and 90°, which are easier to interpret. We selected a sigmoid function to model the behavior of the relationships, defining five zones that represent the influence of the independent variable X on the dependent variable Y, which are described

as follows: (1) in the range [0°, 20°], the influence of X on Y is very low; (2) in the range the influence is low; (3) in the range [40°, 60°], there is a medium influence; (4) in the range [60°, 80°], the influence is high; (5) in the range [80°, 90°], the influence is very high. It is important to mention that the sigmoid function is also applicable to the case of negative influences. For practical reasons, we transform the quantitative relationships between the dependent and independent variables expressed by normalized values (Nv) between 0 to 1 into qualitative values represented by five zones through the sigmoid function depicted in Figure 4. These five zones are described as follows: Zone 1 (range [0, 0.22]), the influence of X in Y is very low; Zone 2 (range [0.22, 0.44]), the influence of X in Y is low; Zone 3 (range [0.44, 0.66]), the influence of X in Y is medium; Zone 4 (range [0.66, 0.88]), the influence of X in Y is high; Zone 5 (range [0.88, 1]), the influence of X in Y is very high. For more details, see Appendix C).

3. Results

3.1. Genetic Diversity of the Three Oak Taxa Hosting Gall-Inducing Insects

Genetic analysis of the *Q. rugosa* × *Q. glabrescens* complex, using eight nuclear microsatellites, showed that *He* presented the following pattern: hybrid (0.669) > *Q. rugosa* (0.637) > *Q. glabrescens* (0.447). The Kruskal–Wallis analysis of variance revealed significant differences among these values, while the Tukey test showed that the highest values, found in the hybrid taxa, differed statistically from both parental species (Table 3). These genetic differences between host plants can lead to changes in morphological, phenological or chemical attributes that constitute the range of resources and conditions that can be exploited by arthropods.

Table 3. Mean (± standard deviation) of genetic diversity (*He*) and concentration of secondary metabolites (SMs) per taxa (*Quercus glabrescens*, *Q. rugosa* and hybrid). Kruskal–Wallis results to determine the effect of oak taxa on genetic diversity and concentration of SMs (rutin, quercetin, caffeic acid, scopoletin, kaempferol glucoside, quercitrin).

Taxa	Genetic Diversity	Rutin	Caffeic Acid	Quercetin	Quercitrin	Kaempferol Glucoside	Scopoletin
	(*He*)	($H_{2,\ 48}$)	($H_{2,\ 48}$)	($H_{2,\ 48}$)	($H_{2,\ 48}$)	($H_{2,\ 48}$)	($H_{2,\ 48}$)
Detection limit (mg/g)		3.701	1.232	3.234	1.348	2.374	1.455
Q. rugosa	0.637 a	4.90 ± 0.20 a	3.59 ± 0.35 a	5.78 ± 0.23 a	16.46 ± 1.53 a	10.65 ± 2.00 ab	8.25 ± 0.96 a
Q. glabrescens	0.447 b	3.37 ± 0.40 b	2.75 ± 0.35 ab	3.55 ± 0.03 b	6.64 ± 0.39 b	5.95 ± 0.51 b	2.62 ± 0.16 b
Hybrid	0.699 c	0.0 c	1.87 ± 0.44 b	4.17 ± 0.12 c	2.44 ± 0.09 c	8.47 ± 0.29 a	1.49 ± 0.24 c
Kruskal–Wallis	73.794 ***	28.852 ***	6.411 *	37.880 ***	38.470 ***	7.639 *	34.455 ***

Different letters show significant differences between taxa: $p < 0.05$. * = $p < 0.05$, *** = < 0.001.

3.2. Qualitative and Quantitative Variation of Secondary Metabolites

A total of six compounds have been identified for this white oak complex, all of which were present in the three taxa analyzed (Table 3). These six compounds were quantitatively characterized (mg/g extract) at the taxa level in the present study. It was found that the concentrations of all the analyzed compounds were significantly different between *Q. rugosa* and *Q. glabrescens*, and that *Q. rugosa* had the highest concentrations of all the metabolites. The most important compounds, in terms of concentration, found in *Q. rugosa* were quercetrin, kaempferol and scopoletin. In the case of *Q. glabrescens*, the most important were quercetrin and kaempferol (Table 3). The concentration of caffeic acid found in the hybrid taxa was different from that found in *Q. rugosa* but not from the concentration found in *Q. glabrescens*; in the case of kaempferol, an inverse pattern was found. With respect to quercetin, its concentration was between the concentrations found in both parental species. The concentrations of quercetrin and scopoletin were below those

found in both parental species. It is worth noting that the most important metabolites, in terms of concentration, found in the hybrid taxa were kaempferol and quercetrin, in that order. The results suggest that (a) the taxa has an effect on the quantitative expression of the secondary metabolites under study and (b) it is possible to identify three patterns of inheritance in the quantitative expression of the hybrid taxa: a dominant inheritance pattern with respect to caffeic acid and kaempferol, since their concentrations were similar to those found in some of the parental species; an intermediate inheritance pattern with respect to quercetrin expression; and a subexpression pattern with respect to quercetrin and scopoletin. Quantitative chemical changes in the host plants of the *Q. glabrescens* × *Q. rugosa* complex can impact herbivorous insects either directly by causing antifeedant or toxic effects upon ingestion or indirectly by attracting their natural enemies. These changes may alter insect herbivory patterns and consequently impact the distribution, abundance and diversity of arthropods.

3.3. Composition of the Community of Gall-Inducing Insects and Their Parasitoids

The canopy arthropod community associated with the *Q. rugosa* × *Q. glabrescens* complex was characterized based on the analysis of 1082 galls belonging to 25 gall wasp (Cynipidae) species. Twenty-two species were recorded in *Q. rugosa*; fifteen in hybrids; and ten in *Q. glabrescens*; all species were grouped into 10 genera (Table 4, Figure 1).

Table 4. List of gall-inducing wasp species and their parasitoids associated with the *Quercus rugosa* × *Q. glabrescens* complex in Central Mexico. P is equal to the presence of the species.

Superfamily	Family	Genus	Species	Host Taxa		
				Q. rugosa	***Q. glabrescens***	**Hybrid**
		Gall wasps				
Cynipoidea	Cynipidae	*Andricus*	*A. sphaericus*	P		P
			A. nievesaldreyi	P	P	
			A. nr georgei	P		P
			A. nr parmula	P		
			A. nr sanchezi	P		P
			A. sp1	P	P	P
			A. nr	P		
		Atrusca	*A. pictor*	P	P	P
			A. grupo bulboides	P	P	P
			A. sp1	P	P	P
			A. sp2	P	P	P
			A. sp3	P	P	P
		Cynips	*C.* sp1		P	P
			C. sp2	P		
			C. sp3			P
			C. sp4	P		P
		Disholcaspis	*D.* sp1	P		P
			D. sp2	P		
		Dros	*D. perlentum*	P		
		Druon	*D. rasfiaum*	P		
		Estriatoandricus	*E. georgei*	P	P	
			E. fornesanus	P		
		Ferum	*F. vitrium*	P		
		Kinseyella	*K. quercusobtusata*			P
		Neuroterus	*N.* sp1	P	P	P
		Parasitoids				
Chalcidoidea	Eulophidae	*Baryscapus*	*Baryscapus* sp.	P		
		Galeopsomia	*Galeopsomia* sp.	P	P	P
	Eurytomidae	*Sycophila*	*Sycophila* sp.	P		
		Eurytoma	*Eurytoma* sp.	P	P	
	Ormyridae	*Ormyrus*	*Ormyrus* sp.	P	P	
	Torymidae	*Torymus*	*Torymus* sp.	P	P	P

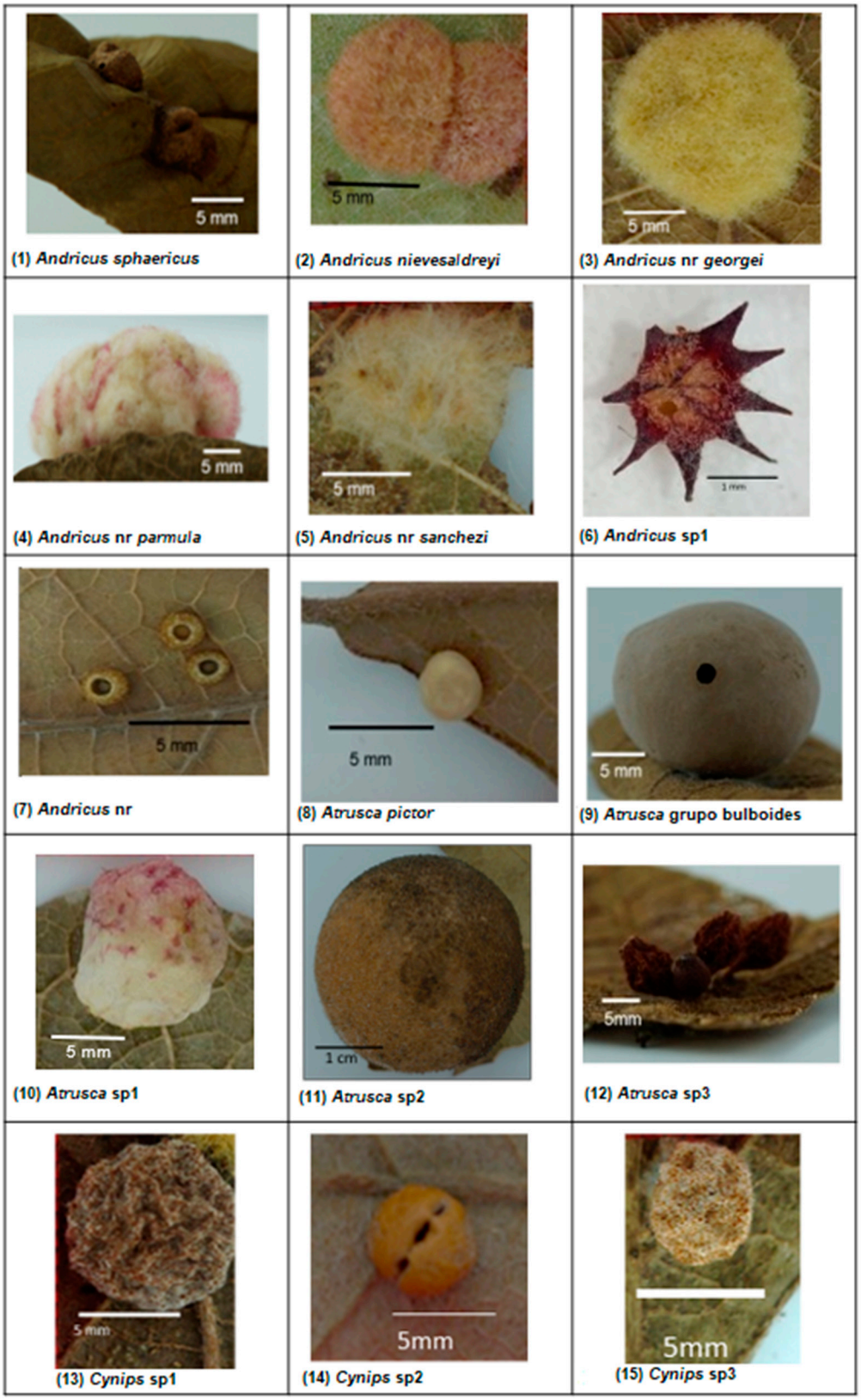

Figure 1. *Cont*.

Figure 1. Gall-inducing wasps found in *Q. glabrescens* × *Q. rugosa* complex.

The most representative genera in terms of the species were *Andricus* (*n* = 12) > *Atrusca* = *Cynips* (*n* = 4) > *Estriatoandricus* (*n* = 2), *Ferum* (*n* = 1) = *Kinseyella* (*n* = 1) = *Neuroterus* (*n* = 1). In terms of abundance, the wasp species with the greatest number of individuals were *Disholcaspis* sp3 (386), *Neuroterus* sp1 (339) and *Andricus georgei* (177). Inquiline wasps of the genus *Synergus* emerged from 2.5% of all the collected galls; these wasps were not included in the analysis. Furthermore, parasitic insects were present in 24.3% of all the collected galls. At the family level, Eulophidae and Eurytomidae were the most representative, with two genera of parasitoids each. For their part, Ormyridae and Torymidae only registered one genera of parasitoids each (Table 4).

3.4. Effect of Genetic Diversity and the Expression of Secondary Metabolites on the Communities of Gall-Inducing Insects and Parasitoids

The results show that *Q. rugosa* had the highest values in all the community parameters analyzed, and these values differed statistically from those reported for *Q. glabrescens* and the hybrid taxa (Table 5). In contrast, there were no significant differences in any of the parameters evaluated between the latter two taxa. Thus, the results of this study suggest the following: (a) an effect of the host taxa on the parameters that characterize the community of gall-inducing insects and parasitoids; (b) a dominant inheritance pattern with respect to the susceptibility of the hybrid taxa to the arthropods associated with its canopy, as this susceptibility did not differ from that found in the parental species *Q. glabrescens*. Our results suggest that the gall-inducing insect community associated with hybrids is similar in structure and diversity to the parental species *Q. glabrescens*.

Table 5. ANOVA results to determine the effect of oak taxa (*Quercus rugosa*, *Q. glabrescens* and hybrid) on abundance, infestation percentage, species richness (mean ± standard deviation) and Shannon–Wiener diversity index (*H'*) of canopy gall-inducing wasps and their parasitoids.

	Abundance	**Infestation (%)**	**Richness**	**Diversity (*H'*)**
Gall wasps				
Q. rugosa	31.85 ± 4.44 a	15.95 ± 2.22 a	6.30 ± 0.51 a	2.492 A
Q. glabrescens	12.35 ± 2.20 b	6.17 ± 1.10 b	1.90 ± 0.39 b	0.821 B
Hybrid	5.35 ± 1.35 b	2.67 ± 0.67 b	2.25 ± 0.39 b	2.429 A
Anova ($F_{2,97}$)	14.979 ***	15.717 ***	29.644 ***	
Parasitoids				
Q. rugosa	6.50 ± 1.15 a	23.16 ± 3.49 a	1.42 ± 0.14 a	0.620 AB
Q. glabrescens	0.70 ± 0.27 b	9.46 ± 3.88 b	0.25 ± 0.07 b	0.905 A
Hybrid	0.75 ± 0.42 b	11.38 ± 5.80 b	0.25 ± 0.09 b	0.362 B
Anova ($F_{2,97}$)	41.359 ***	6.282 *	89.138 ***	

Different small letters show significant differences at $p < 0.05$ (Tukey's honestly significant differences test); different capital letters show significant differences at $p < 0.05$ ([82] δ test). * = $p < 0.05$, *** = < 0.001.

The results of the network analysis show that the *He* of the *Q. glabrescens* × *Q. rugosa* complex had a significant effect on the quantitative expression of four of the six SMs analyzed. Quercetin glucoside, kaempferol glucoside and scopoletin were positively influenced (Figure 2), whereas caffeic acid was negatively influenced (Figure 3).

Furthermore, it was found that *He* had a positive and significant effect on the abundance and richness of gall-inducing insects and parasitoids. However, according to the normalized values (Nvs), the magnitude of the influence of the oak host genetic diversity has the following pattern: abundance and richness of gall insects > abundance and richness of parasitoid insects > SMs (Figure 2). Even so, five out of six SMs analyzed (83.33%) had an influence on some of the parameters of the gall insects community. It was found that scopoletin, quercetin glucoside and caffeic acid had a significant and positive influence on the abundance of these insects. Similarly, rutin and quercetin glucoside positively affected the richness of gall-inducing species. In contrast, quercitrin and rutin had a negative influence on the abundance of gall insects (Figures 2 and 3). It is worth noting that, in terms of normalized values (Nvs), the influence of the SMs showed the following pattern: abundance > gall insect richness, regardless of direction. Finally, it was found that only 33.33% (two out of six) of the SMs analyzed had an influence on the parameters that characterize the parasitoid insect community. It was found that quercitrin had a positive influence on the abundance and richness of parasitoids, while scopoletin negatively affected both parameters (Figures 2 and 3).

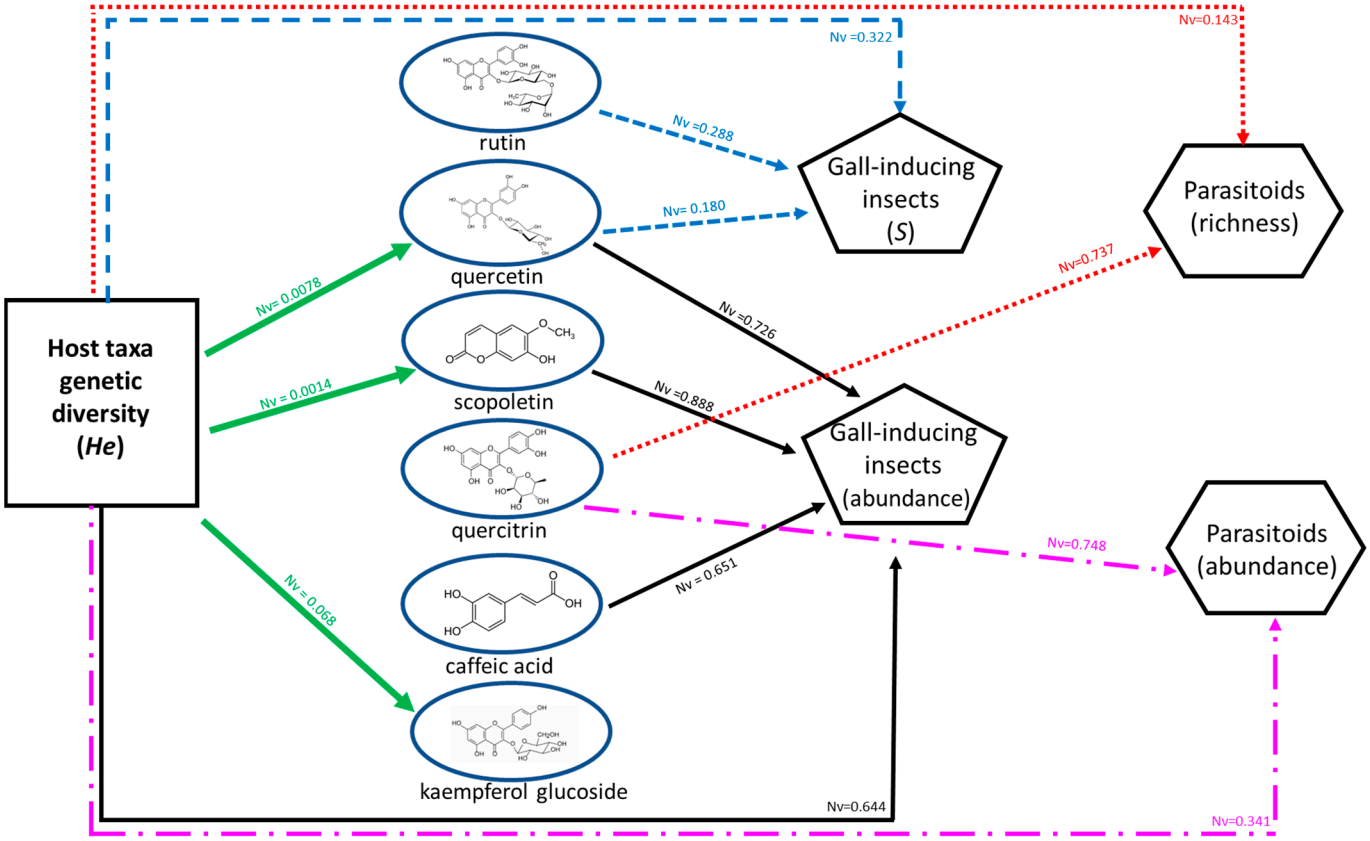

Figure 2. Network related to the positive influence between host oak [genetic diversity (*He*), secondary metabolites (rutin, caffeic acid, quercetin glucoside, quercitrin, kaempferol glucoside, scopoletin)] and the richness (*S*) and abundance of canopy gall-inducing wasps and their parasitoids. Nv = normalized value (from 0 to 1), where (1) in the range [0.0–0.22], the influence is very low; (2) in the range [0.22–0.44], the influence is low; (3) in the range [0.44–0.66], there is a medium influence; (4) in the range [0.66–0.88], the influence is high; and (5) in the range [0.88–1], the influence is very high.

The network results show that the range of normalized values [0.66–0.88] is of interest (Figure 4). This is the case for the following relationships depicted in the network related to positive influences, all of them converging in the abundance of gall-inducing wasps: (a) quercetin glucoside → abundance of gall-inducing wasps = 0.726, (b) scopoletin → abundance of gall-inducing wasps = 0.888, (c) caffeic acid → abundance of gall-inducing wasps = 0.651 (this value is very close to the high-influence zone), (d) *He* → abundance of gall-inducing insects = 0.644 (this value is very close to the high-influence zone) (Figure 2). Derived from these relationships, we can conclude that three SMs contribute importantly to the abundance of gall-inducing wasps. This condition gives special importance to the role of SMs in the abundance of gall-inducing wasps.

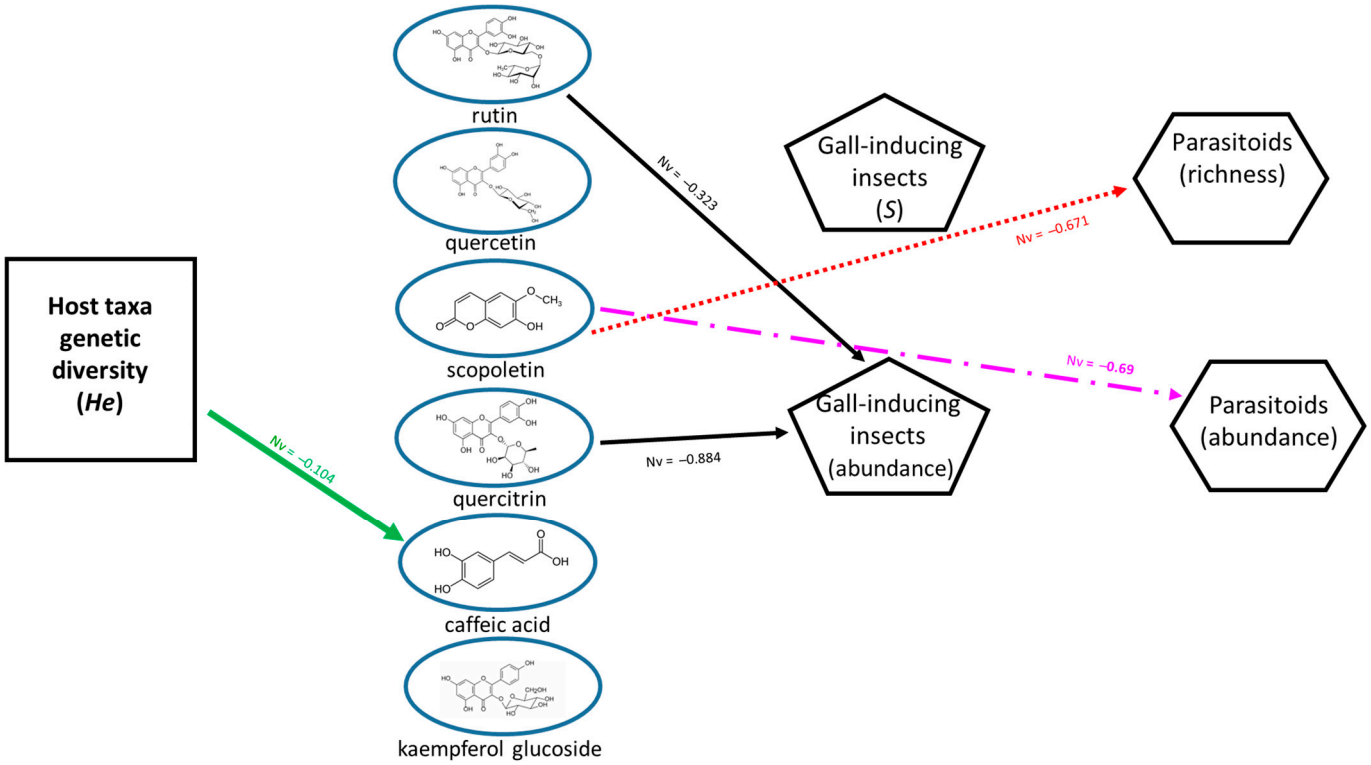

Figure 3. Network related to the negative influence between host oak [genetic diversity (*He*), secondary metabolites (rutin, caffeic acid, quercetin glucoside, quercitrin, kaempferol glucoside, scopoletin)] and the richness (*S*) and abundance of canopy gall-inducing wasps and their parasitoids. Nv = normalized value (from 0 to 1), where (1) in the range [0.0–0.22], the influence is very low; (2) in the range [0.22–0.44], the influence is low; (3) in the range [0.44–0.66], there is a medium influence; (4) in the range [0.66–0.88], the influence is high; and (5) in the range [0.88–1], the influence is very high.

In the case of the network of negative influences, the influence of SMs scopoletin on the richness and abundance of gall parasitoids is noteworthy. The value of Nv in these relationships is located in the zone of high influence. The negative influence of the quercitrin metabolite in the abundance of gall-inducing wasps is specially highlighted, because the value of the Nv is very close to the zone of very high influence (Figure 3). These findings suggest that the variation in SMs concentrations in the host plants of the *Q. glabrescens* × *Q. rugosa* complex is influenced by genetic factors, and that plant chemistry serves as an intermediary connecting plant genes to the herbivore community and its associated parasitoids.

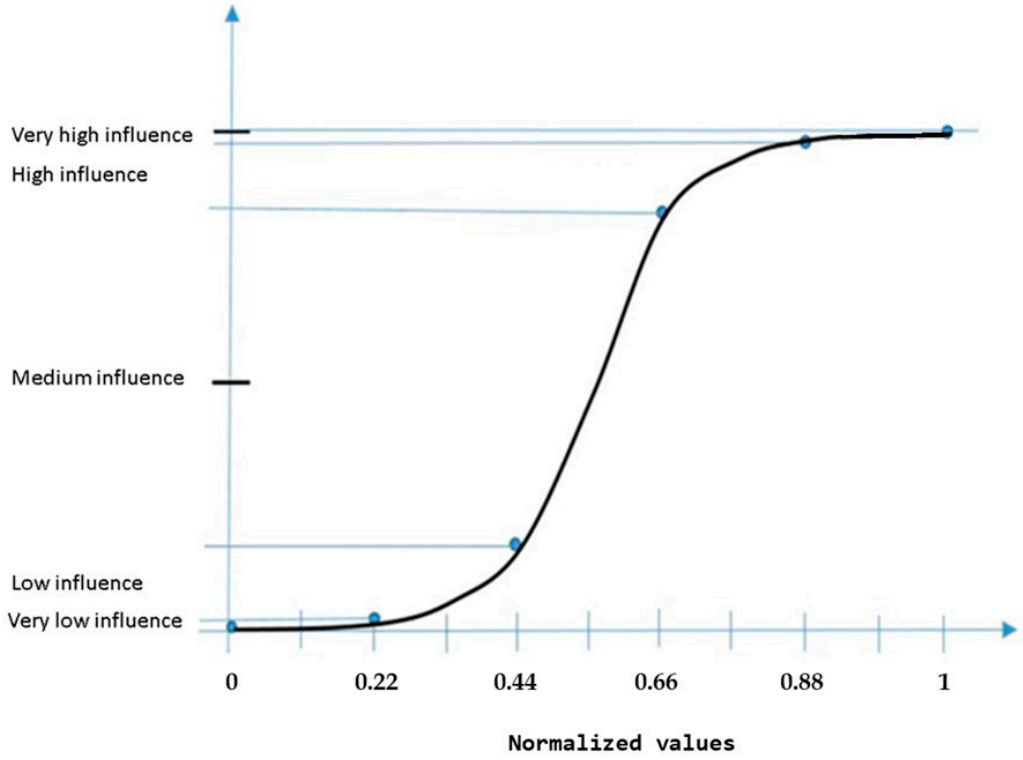

Figure 4. Function that models the behavior of the relationships depicted in the network related to the influences. The *X* axis shows the normalized values (Nvs) between 0 and 1. The sigmoid function models the behavior of the relationships through qualitative values, which are easier to interpret. We define five zones that represent the influence of the independent variable *X* on the dependent variable Y, which are (1) in the range [0–0.22], the influence of *X* on *Y* is very low; (2) in the range [0.22–0.44], the influence is low; (3) in the range [0.44–0.66], there is a medium influence; (4) in the range [0.66–0.88], the influence is high; and (5) in the range [0.88–1], the influence is very high. See Appendix C for more details.

4. Discussion

The present study analyzed simultaneously the impact of genetic diversity (*He*, modified by hybridization events) and the expression (quantitatively) of six foliar SMs (flavonoid and coumarin) on the arthropod communities of two trophic levels associated with a white oak canopy complex: gall–inducing wasps (herbivores) and their associated parasitoids (predators).

4.1. Genetic Diversity and Secondary Metabolite Expression

Recent studies have reported that the *He* of some oak taxa oscillates between 0.25 and 0.88 [26,85–87]. In the present work, we found that *Q. rugosa*, *Q. glabrescens* and their hybrids presented *He* values of 0.43, 0.47 and 0.69, respectively, data that are in accordance with previous reports. Moreover, our results showed that the hybrid taxa presented the highest *He* values in comparison with both parental taxa. This result is consistent with other reports for oak populations where hybrids have been detected [26,88]. It has been suggested that hybrid taxa constitute new genetic variants that result from the pool genetic combination of their parental taxa, a condition that is expressed in a higher level of genetic diversity [26].

In general, it has been reported that the expression of SMs in plants, including oak species, has an important genetic component [29,89]; we analyzed flavonoid and coumarin expression, considering the existing information about their expression and inheritability [90] that suggests that both parameters are strongly determined by genetic

factors [13,91–93]. In this context, Klaper et al. [94] found that at least seven phenolic compounds have a high probability of being inheritable in *Q. laevis*. Specifically, the authors reported that the expression of these compounds in this oak species is the result of an additive genetic variability pattern.

In a previous study, Castillo-Mendoza et al. [43] documented the presence of nine SMs (eight phenolic compounds and one coumarin) in the *Q. glabrescens* × *Q. rugosa* complex. Following the same methodology, it was possible to identify two more compounds, kaempferol glucoside (phenolic compound) and scopoletin (coumarin). The quantitative analysis of the caffeic acid, quercetin glucoside, rutin and quercitrin (characterized by Castillo-Mendoza et al. [43]) and both compounds identified in the present work revealed that hybridization between *Q. glabrescens* and *Q. rugosa* affects the quantitative expression of the SMs analyzed. Particularly, it was found that the hybrid taxa show three patterns of SMs expression: dominant, intermediate and subexpression. This finding is in accordance with the scientific literature, where the first two patterns are the most common in plants [38,39]. Also, Rehill et al. [95], using the previously mentioned methodology, found that the heritability of phenolic compounds is controlled by dominant genes, although the development strategies differ between hybrid and parental taxa.

The subexpression pattern found for quercitrin and scopoletin can be explained as follows: (a) the low concentration of these compounds on foliar tissue, that makes it undetectable (optimal defense theory [96]); (b) hybridization may modify the metabolic route that drives to its quantitative expression [97]; and (c) point mutations in biosynthetic genes because of interspecific gene flow. Finally, Cheynier et al. [98] mentioned that phenolic compound expression is produced mainly through changes in the transcription rate of biosynthetic genes. Such factors are highly sensitive to changes in their components. Hence, if hybridization processes alter any of the basic components of the metabolic route, this change may in turn repress the expression of the final product, such as in the case of rutin in our study.

4.2. Characterization of Gall-Inducing Insect Community and Their Parasitoids

In the present study, we documented the highest species richness of gall-inducing insects associated with *Q. rugosa* and the lowest species richness associated with *Q. glabrescens*. This pattern may be explained by their contrasting geographic range distribution in Mexico. For example, *Q. rugosa* is distributed along 27 Mexican states [99], suggesting the environmental heterogeneity in which this species distributes itself, resulting in its high phenotypic plasticity. It has been documented that a higher phenotypic plasticity of oak host canopies may result in more resources and conditions, creating a higher number of microhabitats for gall-inducing wasps [33]. Moreover, in accordance with the international oak society, *Q. rugosa* participates in hybridization events with at least five other white oak species not including *Q. glabrescens*. These events may contribute to a wider range of resources and conditions that *Q. rugosa* is offering to its canopy insects. On the contrary, *Q. glabrescens* is distributed along nine Mexican states and at altitudes higher than 2500 m; therefore, this may limit gall-inducing wasp establishment, due to a reduction in their colonization areas. Moreover, due to the environmental and geographical conditions in which this oak species distributes itself, its associated insects should have particular characteristics that allows them to establish themselves [100]. Pascual-Alvarado et al. [101] did not find galls associated with *Q. glabrescens*. Finally, a pattern of dominant heritage was documented for the hybrid taxa because the analyzed parameters for both gall-inducing insects and their parasitoids did not differ from the parameters reported for the parental species *Q. glabrescens*. This result agrees with reports where this genetic pattern is the most common found in plants [38,39].

Another result is that we found six parasitoid genera (grouped in four families) associated with 18 gall species. For example, Serrano-Muñoz [102] reported the presence of nine parasitoid genera associated with six gall wasp genera (in 17 oak species). Also, Valencia-Cuevas et al. [9] registered the presence of 10 genera associated with 18 gall species (in *Q. castanea*). It has been suggested that three key factors influence parasitoid community structure: (1) spatiotemporal niche traits define the distribution of hosts in space (oak taxon galled, location of the gall on the oak) and time (season and duration of development) that determine the likelihood of recognition by parasitoids; (2) resource traits define the quality of the host resource per gall (number of hosts per gall, host size) potentially available to parasitoids; and (3) gall morphology traits capture variation in the structure of the gall tissues parasitoids must penetrate to access the host resources, acting as direct defenses against particular natural enemies [103]. These groups of traits affect parasitoid success in host detection (spatiotemporal niche) and host exploitation (resource, morphology), respectively [104]. Also, these results highlight the need for more sampling. Specifically, in this work we found a positive and significant relationship between gall-inducing insects and parasitoid richness, a fact that suggests that this variation in parasitoid species richness may be directly related to the resources that gall species offer (the number of hosts per gall, host size), as mentioned. In the future, it would be useful to test these hypotheses.

In this study, we found three parasitoid genera (*Torymus*, *Ormyrus* and *Eurytoma*), in addition to *Sycophila*, which has also been reported in red oaks [9,105], a fact that may suggest that these insects may be generalists, independently of if they establish on red or white oaks. In the case of *Baryscapus* and *Galeopsomyia,* they were found only established on white oaks [105], supposing a certain degree of specificity.

4.3. Effect of Genetic and Chemical Diversity on Gall-Inducing Insect Community and Their Parasitoids

The network analysis showed the magnitude and direction of the relationships between genetic diversity (*He*), SMs (flavonoids) and the gall-inducing insect community and their parasitoids (abundance and richness) associated with the *Q. glabrescens* × *Q. rugosa* complex. Specifically, we found that *He* had a positive effect on tree SMs: quercetin glucoside, kaempferol glucoside, scopoletin. In contrast, a negative influence was found on caffeic acid. These results are in agreement with studies that report that the expression and heritability of these SMs in plants are strongly determined by genetic factors [91–93]. In the case of caffeic acid, this compound is a precursor of different flavonoids and coumarins (the Shikimic acid pathway [105,106]), and it is not a final product of this route. Hence, we can suggest that the decrease in caffeic acid concentration (negative influence), along with the increase in the genetic diversity levels of the analyzed taxa, may be because this compound is being used as a precursor for quercetin glucoside, scopoletin and kaempferol glucoside production, a fact that could also explain the increase in these compounds (positive influence) as genetic diversity increases. Also, we found that *He* had a positive and significant effect on the abundance and richness of gall-inducing insects. Similar results have been registered in phytophagous insect communities associated with different plant species (e.g., poplars [16]; eucalyptus [107]; willows [108]; and oaks [79]). For example, Valencia-Cuevas et al. [9] found that the genetic diversity of the oak host species *Q. castanea* had a positive and significant influence on richness and endophagous insect density (Cynipidae). An increase in host plant genetic diversity can enhance their architectural complexity and nutritional quality [109], which subsequently favors a greater abundance of herbivorous insects [110] due to an increase in resources and conditions that they can employ [8]. Additionally, higher genetic diversity can alter the arthropod community structure by affecting the host plant's resistance or susceptibility to herbivores, as well as the herbivores' ability to recognize the host plant as a suitable host [111]. Moreover, it has been reported that

primary productivity increases as genetic diversity also increases, so a higher number of individuals and arthropod species can be supported by plants with higher levels of genetic diversity [112].

Additionally, we found that the positive effect of oak host genetic diversity scaled up to the next trophic level: the richness and abundance of insects and parasitoids. It has been proposed that direct effects that have a genetic basis in plants also influence the next trophic level [32]. Under this scenario, we expected that richness and gall insect abundance would be influenced directly by the genetic traits of the host plant, but also those genetic traits would have an influence on the parasitoid insect community. This last hypothesis was supported by our results, since the network analysis evidenced that the host taxa *He* had a positive and significant influence on two parameters of the parasitoid insect community. These last results are in accordance with Bailey et al. [113], Johnson [114] and Valencia-Cuevas et al. [9], who suggest that genetic variation in plants may be a determinant factor that regulates herbivore population dynamics and interactions between plants, herbivores and parasitoids.

Although host genetic diversity had a positive and significant influence on herbivores and their parasitoid community, in agreement with the Nv values, the host genetic diversity influence pattern found in our study is as follows: abundance and richness of gall-inducing insects > abundance and richness of parasitoid insects. This pattern may be explained because herbivores obtain resources directly from their host plants [115]. So, genetic and chemical changes in host plants will have direct consequences and a greater magnitude of influence on their associated herbivores than on parasitoid communities. Also, we found that the abundance and richness of parasitoid insects were affected positively. This last result is in accordance with Koricheva and Hayes [116], who evidenced through a meta-analysis the positive effects of the genetic diversity levels of the host plant on predator and parasitoid arthropods.

In this study, we detected quantitative chemical changes in the host plants of the *Q. glabrescens* × *Q. rugosa* complex. This condition can impact herbivorous insects either directly by causing antifeedant or toxic effects upon ingestion or indirectly by attracting their natural enemies [117]. These changes may alter insect herbivory patterns and consequently impact the distribution, abundance and diversity of these arthropods and their associated parasitoids. An influence analysis also showed the magnitude and direction of the SMs effect on arthropods associated with the *Q. glabrescens* × *Q. rugosa* complex. In terms of normalized values (Nvs), SMs influence registered the next pattern: abundance and richness of gall-inducing insects > abundance and richness of parasitoid insects, independently of the direction. This pattern may be explained when considering that herbivores have a more direct influence on their host plant attributes, such as SMs, in comparison to parasitoid insects that depend more on their direct resources, which are these herbivores. This is also supported by the fact that 83.33% of the SMs analyzed had an influence on gall-inducing insects and only 33.33% affected parasitoid insects. Stahl and co-workers [118] documented that the interaction of herbivore–plant is highly dependent on mechanisms that are related to SMs, specifically in toxification–detoxification mechanisms. Parasitoids can be attracted by the emission of volatile compounds that enable them to detect potential prey, a fact that could indirectly regulate the success of herbivore attacks in some species [119,120]. We do not know that other cues are at play in this system; however, in the literature it has been reported that in addition to olfactory stimulus, parasitoid searching and host location is influenced by other external stimuli such as visual ones (e.g., shoots containing developing galls or free of galls but with expanded leaves), tactile one (vibrational sounding) and even cues of CO_2 (that signal locations of actively respiring gall larvae/pupae) [121,122]. Also, our analysis showed that caffeic acid, scopoletin and quercetin glucoside had a positive and

significant influence on gall insect abundance. In general, it is known that caffeic acid may favor scopoletin expression [123], which has antifungal and antibacterial properties [124]. For example, scopoletin is a phenolic coumarin that can be isolated from various plant species [125], and it has been proposed as an important phytoalexin against pathogens [124]. For wild tobacco (*Nicotiana attenuata*), it has been documented that scopoletin possessed antifungal activity against the necrotrophic fungus *Alternaria alternata*, in vitro and in vivo conditions [126]. In this context, it has been reported that for cynipids the fungal mortality is ecologically significant, and the host flavonoids (as tannins) may serve a defensive function, helping to reduce the levels of fungal infestation, as reported for the cynipid gall wasp *Dryocosmus dubiosus* associated with *Q. agrifolia* and *Q. wislizenii* [127]. So, this metabolite may increase the resistance to fungi attacks, enabling gall wasps to complete their life cycle; however, to probe this hypothesis, it would be necessary to carry out studies to know whether the gall insects feeding on these oaks containing these compounds. For the case of quercetin glucoside, it has been reported that this is a metabolite that can be a feeding stimulant for herbivore insects [128]. This response was observed in the larvae of the silkworm *Bombyx mori* fed with the foliar tissue of its host the mulberry tree, *Morus alba* [129], and in the Western corn root worm *Diabrotica virgifera*, which feeds on the pollen of the sunflower *Helianthus annuus* L. [130].

Quercitrin also had a positive and statistically significant effect on richness and parasitoid abundance. Possibly, the expression of this particular SM may be part of the indirect defense of the plant, resulting in the attraction of different parasitoid species and/or predators [131,132].

So, an increase in the concentration of this SM may help oak host species to reduce the virulence of gall wasps, favoring the abundance and diversity of parasitoid insects. Similarly, rutin and quercetin glucoside positively affected gall species richness. In contrast, quercitrin and rutin had a negative influence on gall-inducing insect abundance. In this sense, it is important to mention the biological properties of these compounds; it has been reported that rutin and quercetin glucoside had a positive impact on the richness of gall-inducing insects, probably because they act as feeding cues or stimulants influencing insect feeding behavior, favoring more species on the host plant [133]. In contrast, rutin and quercitin had a negative influence on gall wasp abundance, perhaps because these SMs affect the growth, survival and development of herbivores, being lethal at high concentrations [134,135].

On the other hand, caffeic acid has an influence on the feeding, growth and survival rates of generalist insects and on the reproductive structures of fungi and bacteria (bacteriostatic or bactericidal effects) [136–138]. The last mentioned effects may have a negative impact on secondary fauna (mites, spiders and different predators) and on gall decomposition rates, enabling the development of more diverse communities with a higher abundance, in comparison to those that do not express this particular SM.

Finally, scopoletin had a negative impact on richness and on parasitoid abundance. Diverse studies have documented that this SM reduces female fecundity and longevity [107], a fact that suggests that the negative effects have an impact only on parasitoids, and as documented earlier, scopoletin impacts positively on gall wasp abundance, possibly because of the elimination of their natural predators. However, more experiments are needed to increase our knowledge about how insects not only perceive these metabolites, but also how they utilize them. This would help us understand more completely the role they play in plant–insect interactions.

5. Conclusions

The results obtained in the present study show that all the aims of the present study were addressed. Specifically, that an increase in oak host genetic diversity as a result of hybridization events influences the expression of SMs with high heritability rates, as well as having negative effects on the herbivore and parasitoid communities of host plants. These findings demonstrate the importance that genetic and secondary chemical diversity has on the plant–herbivore–parasitoid interactions. Hence, the loss of the genetic diversity levels of host plants may result in a loss of arthropod species along trophic levels, a fact that comprises the ecosystem function. Moreover, hybridization events between *Q. glabrescens* and *Q. rugosa* promoted quantitative differences in the pattern of SM expression in their hybrids. Regarding the construction of networks related to these positive and negative influences, they allowed the building of influence pathways to facilitate the analysis of the influence of genetic diversity and host plant SMs on the richness and abundance of gall inductors and their parasitoids.

Author Contributions: Conceptualization, E.T.-S.; methodology, E.C.-M. and A.Z.; validation, E.T.-S.; formal analysis, E.T.-S. and F.R.-Q.; investigation, E.C.-M. and L.V.-C.; resources, E.T.-S. and P.M.-G.; data curation, E.C.-M., J.P.-V. and M.S.-M.; writing—original draft preparation, E.C.-M.; writing—review and editing, E.T.-S., L.V.-C. and P.M.-G.; supervision, E.T.-S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

UV spectra, chemical structure and chromatogram of phenolic compounds: quercetin-3-O-rutinoside (= rutin) (1), caffeic acid (2), quercetin-3-O-glucoside (= quercetin) (3), kaempferol-3-O-glucoside (= kaempferol glucoside) (4), quercetin-3-O-rhamnoside (= quercitrin) (5) and scopoletin (6) present in *Q. rugosa*, hybrids and *Q. glabrescens*.

Appendix B

Retention time in minutes of the phenolic compounds characterized in three oak taxa and the corresponding commercial reference (Sigma-Aldrich).

Appendix C

Conceptual development of the model. The way of quantifying the causal relationship between two variables

References

1. Murdoch, W.W.; Evans, F.C.; Peterson, C.H. Diversity and pattern in plants and insects. *Ecology* **1972**, *53*, 819–829. [CrossRef]
2. Pérez-López, G.; González-Rodríguez, A.; Oyama, K.; Cuevas-Reyes, P. Effects of plant hybridization on the structure and composition of a highly rich community of cynipid gall wasps: The case of the oak hybrid complex *Quercus magnoliifolia* x *Quercus resinosa* in Mexico. *Biodivers. Conserv.* **2016**, *25*, 633–651. [CrossRef]
3. Shuster, S.M.; Lonsdorf, E.V.; Wimp, G.M.; Bailey, J.K.; Whitham, T.G. Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution* **2006**, *60*, 991–1003. [CrossRef]
4. Harvey, J.A.; Van Dam, N.M.; Gols, R. Interactions over four trophic levels: Foodplant quality affects the development of a hyperparasitoid as mediated through an herbivore and its primary parasitoid. *J. Anim. Ecol.* **2003**, *72*, 520–531. [CrossRef]
5. Preszler, R.W.; Boecklen, W.J. A three-trophic-level analysis of the effects of plant hybridization on a leaf-mining moth. *Oecologia* **1994**, *100*, 66–73. [CrossRef]
6. Crutsinger, G.M.; Collins, M.D.; Fordyce, J.A.; Gompert, Z.; Nice, C.C.; Sanders, N.J. Plant Genotypic Diversity Predicts Community Structure and Governs an Ecosystem Process. *Science* **2006**, *313*, 966–968. [CrossRef]
7. Wimp, G.M.; Wooley, S.; Bangert, R.K.; Young, W.P.; Martinsen, G.D.; Keim, P.; Rehill, B.; Indroth, R.L.L.; Whitham, T.G. Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. *Mol. Ecol.* **2007**, *16*, 5057–5069. [CrossRef]
8. Tovar-Sánchez, E.; Oyama, K. Effect of hybridization of the *Quercus crassifolia* × *Quercus crassipes* complex on the community structure of endophagous insects. *Oecologia* **2006**, *147*, 702–713. [CrossRef] [PubMed]
9. Valencia-Cuevas, L.; Mussali-Galante, P.; Cano-Santana, Z.; Pujade-Villar, J.; Equihua-Martínez, A.; Tovar-Sánchez, E. Genetic variation in foundation species governs the dynamics of trophic interactions. *Curr. Zool.* **2018**, *64*, 13–22. [CrossRef] [PubMed]
10. Pichersky, E.; Gang, D.R. Genetics and biochemistry of secondary metabolites in plants: An evolutionary perspective. *Trends Plant Sci.* **2000**, *5*, 439–445. [CrossRef] [PubMed]
11. Coq-Etchegaray, D.; Bernillon, S.; Le-Provost, G.; Kremer, A.; Ducousso, A.; Lalanne, C.; Bonne, F.; Moing, A.; Plomion, C.; Brachi, B. Extensive variation of leaf specialized metabolite production in sessile oak (*Quercus petraea*) populations is to a large extent genetically determined but not locally adaptive. *bioRxiv* **2023**. [CrossRef]
12. Robinson, K.M.; Ingvarsson, P.K.; Jansson, S.; Albrectsen, B.R. Genetic variation in functional traits influences arthropod community composition in aspen (*Populus tremula* L.). *PLoS ONE* **2012**, *7*, e37679. [CrossRef]
13. Caseys, C.; Stritt, C.; Glauser, G.; Blanchard, T.; Lexer, C. Effects of hybridization and evolutionary constraints on secondary metabolites: The genetic architecture of phenylpropanoids in European *Populus* species. *PLoS ONE* **2015**, *10*, e0128200. [CrossRef] [PubMed]
14. Ehrlich, P.R.; Raven, P.H. Butterflies and plants: A study in coevolution. *Evolution* **1964**, *18*, 586–608. [CrossRef]
15. Mutikainen, P.; Walls, M.; Ovaska, J.; Keinänen, M.; Julkunen-Tiitto, R.; Vapaavuori, E. Herbivore resistance in *Betula pendula*: Effect of fertilization, defoliation, and plant genotype. *Ecology* **2000**, *81*, 49–65. [CrossRef]
16. Whitham, T.G.; Bailey, J.K.; Schweitzer, J.A.; Shuster, S.M.; Bangert, R.K.; LeRoy, C.J.; Lonsdorf, E.V.; Allan, G.J.; DiFazio, S.P.; Potts, B.M.; et al. A framework for community and ecosystem genetics: Form genes to ecosystems. *Nature* **2006**, *7*, 510–523. [CrossRef]
17. Wimp, G.M.; Martinsen, G.D.; Floate, K.D.; Bangert, R.K.; Whitham, T.G. Plant genetic determinants of arthropod community structure and diversity. *Evolution* **2005**, *59*, 61–69. [CrossRef]
18. Maldonado-López, Y.; Cuevas-Reyes, P.; González-Rodríguez, A.; Pérez-López, G.; Acosta-Gómez, C.; Oyama, K. Relationships among plant genetics, phytochemistry and herbivory patterns in *Quercus castanea* across a fragmented landscape. *Ecol. Res.* **2015**, *30*, 133–143. [CrossRef]
19. Bangert, R.K.; Allan, G.J.; Turek, R.J.; Wimp, G.M.; Meneses, N.; Martinsen, G.D.; Keim, P.; Whitham, T.G. From genes to geography: A genetic similarity rule for arthropod community structure at multiple geographic scales. *Mol. Ecol.* **2006**, *15*, 4215–4228. [CrossRef]
20. Cavender-Bares, F.; Fallon, B.; González-Rodríguez, A.; Hipp, A.L.; Hoerner, F.; Kaproth, M.; Manos, P.S.; Meireles, J.; McVay, J.; Pearse, I. Diversity, distribution and ecosystem services of the north American oaks. *Int. Oaks* **2016**, *27*, 37–48.
21. Govaerts, R.; Frodin, D.G. *World Checklist and Bibliography of Fagales (Betulaceae, Corylaceae, Fagaceae and Ticodendraceae)*; Royal Bot Gardens; Kew: Richmond, VA, USA, 1998.
22. Valencia, A.S. Diversidad del género *Quercus* (Fagaceae) en México. *B. Soc. Bot. Mex.* **2004**, *75*, 33–53. [CrossRef]
23. Bargali, K.; Joshi, B.; Bargali, S.S. Diversity within oaks. *Int. Oaks* **2014**, *25*, 7–70.
24. Skarpaas, O.; Blumentrath, S.; Evju, M.; Sverdrup-Thygeson, A. Prediction of biodiversity hotspots in the Anthropocene: The case of veteran oaks. *Ecol. Evol.* **2017**, *7*, 7987–7997. [CrossRef] [PubMed]
25. Kremer, A.; Abbott, A.G.; Carlson, J.E.; Manos, P.S.; Plomion, C.; Sisco, P.; Staton, M.E.; Ueno, S.; Vendramin, G.G. Genomics of Fagaceae. *Tree Genet. Genomes* **2012**, *8*, 583–610. [CrossRef]

26. Valencia-Cuevas, L.; Piñero, D.; Mussali-Galante, P.; Valencia-Ávalos, S.; Tovar-Sánchez, E. Effect of a red oak species gradient on genetic structure and diversity of *Quercus castanea* (Fagaceae) in Mexico. *Tree Genet. Genomes* **2014**, *10*, 641–652. [CrossRef]
27. Peñaloza-Ramírez, J.M.; González-Rodríguez, A.; Mendoza-Cuenca, L.; Caron, H.; Kremer, A.; Oyama, K. Interspecific gene flow in a multispecies oak hybrid zone in the Sierra Tarahumara of Mexico. *Ann. Bot.* **2010**, *105*, 389–399. [CrossRef]
28. Tovar-Sánchez, E.; Oyama, K. Natural hybridization and hybrid zones between *Quercus crassifolia* and *Quercus crassipes* (Fagaceae) in Mexico: Morphological and molecular evidence. *Am. J. Bot.* **2004**, *91*, 1352–1363. [CrossRef] [PubMed]
29. Yarnes, C.T.; Boecklen, W.; Tuominen, K.; Salminen, J.P. Hybridization affects seasonal variation of phytochemical phenotypes in an oak hybrid complex (*Quercus gambelii* × *Quercus grisea*). *Int. J. Plant Sci.* **2008**, *169*, 567–578. [CrossRef]
30. López-Caamal, A.; Tovar-Sánchez, E. Genetic, morphological, and chemical patterns of plant hybridization. *Rev. Chil. Hist. Nat.* **2014**, *87*, 16. [CrossRef]
31. Whitham, T.G.; Gehring, C.A.; Lamit, L.J.; Wojtowicz, T.; Evans, L.M.; Keith, A.R.; Smith, D.S. Community specificity: Life and afterlife effects of genes. *Trends Plant Sci.* **2012**, *17*, 271–281. [CrossRef]
32. Crutsinger, G.M. A community genetics perspective: Opportunities for the coming decade. *New Phytol.* **2016**, *210*, 65–70. [CrossRef]
33. Valencia-Cuevas, L.; Tovar-Sánchez, E. Oak canopy arthropod communities: Which factors shape its structure? *Rev. Chil. Hist. Nat.* **2015**, *88*, 15. [CrossRef]
34. Becerra, J.X. On the factors that promote the diversity of herbivorous insects and plants in tropical forests. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 6098–6103. [CrossRef] [PubMed]
35. Irchhaiya, R.; Kumar, A.; Yadav, A.; Gupta, N.; Kumar, S.; Gupta, N.; Kumar, S.; Yadav, V.; Prakash, A.; Gurjar, H. Metabolites in plants and its classification. *Int. J. Pharm. Sci.* **2014**, *4*, 287–305.
36. Arnold, M.L.; Martin, N.H. Hybrid fitness across time and habitats. *Trends Ecol. Evol.* **2010**, *25*, 530–536. [CrossRef] [PubMed]
37. Rieseberg, L.H.; Ellstrand, N.C. What can morphological and molecular markers tell us about plant hybridization. *Crit. Rev. Plant Sci.* **1993**, *12*, 213–241. [CrossRef]
38. Cheng, D.; Vrieling, K.; Klinkhamer, P.G. The effect of hybridization on secondary metabolites and herbivore resistance: Implications for the evolution of chemical diversity in plants. *Phytochem. Rev.* **2011**, *10*, 107–117. [CrossRef] [PubMed]
39. Orians, C.M. The effects of hybridization in plants on secondary chemistry: Implications for the ecology and evolution of plant-herbivore interactions. *Am. J. Bot.* **2000**, *87*, 1749–1756. [CrossRef]
40. Sepúlveda-Jiménez, G.; Porta-Ducoing, H.; Rocha-Sosa, M. La participación de los metabolitos secundarios en la defensa de las plantas. *Rev. Mex. Fitopatol.* **2003**, *21*, 355–363.
41. Glassmire, A.E.; Jeffrey, C.S.; Forister, M.L.; Parchman, T.L.; Nice, C.C.; Jahner, J.P.; Wilson, J.S.; Walla, T.R.; Richards, L.A.; Smilanich, A.M.; et al. Intraspecific phytochemical variation shapes community and population structure for specialist caterpillars. *New Phytol.* **2016**, *212*, 208–219. [CrossRef]
42. Usié, A.; Simões, F.; Barbosa, P.; Meireles, B.; Chaves, I.; Gonçalves, S.; Folgado, A.; Almeida, M.H.; Matos, J.; Ramos, A.M. Comprehensive analysis of the cork oak (*Quercus suber*) transcriptome involved in the regulation of bud sprouting. *Forests* **2017**, *8*, 486. [CrossRef]
43. Castillo-Mendoza, E.; Salinas-Sánchez, D.; Valencia-Cuevas, L.; Zamilpa, A.; Tovar-Sánchez, E. Natural hybridisation among *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata* (Fagaceae): Microsatellites and secondary metabolites markers. *Plant Biol.* **2018**, *21*, 110–121. [CrossRef] [PubMed]
44. Moctezuma, C.; Hammerbacher, A.; Heil, M.; Gershenzon, J.; Méndez-Alonzo, R.; Oyama, K. Specific polyphenols and tannins are associated with defense against insect herbivores in the tropical oak *Quercus oleoides*. *J. Chem. Ecol.* **2014**, *40*, 458–467. [CrossRef] [PubMed]
45. Tovar-Sánchez, E.; Castillo-Mendoza, E.; Valencia-Cuevas, L.; Serrano-Muñoz, M.; Mussali-Galante, P. Proximal and evolutionary factors that influence arthropod community structure associated to vascular plants. In *Focus on Arthropods Research*; Nova: New York, NY, USA, 2018; pp. 170–231.
46. Stone, G.N.; Schönrogge, K.; Atkinson, R.J.; Bellido, D.; Pujade-Villar, J. The population biology of oak gall wasp (Hymenoptera: Cynipidae). *Annu. Rev. Entomol.* **2002**, *47*, 633–668. [CrossRef]
47. Abrahamson, W.G.; Hunter, M.D.; Melika, G.; Price, P.W. Cynip gall-wasp communities correlate with oak chemistry. *J. Chem. Ecol.* **2003**, *29*, 209–223. [CrossRef] [PubMed]
48. Raman, A.; Schaefer, C.W.; Withers, T.W. *Biology, Ecology, and Evolution of Gall-Inducing Arthropods*; Science Publishers, Inc.: Plymouth, UK, 2005.
49. Hayward, A.; Stone, G.N. Oak gall wasp communities: Evolution and ecology. *Basic. Appl. Ecol.* **2005**, *6*, 435–443. [CrossRef]
50. Ode, P.J. Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* **2006**, *51*, 163–185. [CrossRef]
51. López-Caamal, A.; Reyes-Chilpa, R.; Tovar-Sánchez, E. Hybridization between *Tithonia tubaeformis* and *T. rotundifolia* (Asteraceae) evidenced by nSSR and secondary metabolites. *Plant Syst. Evol.* **2018**, *304*, 313–326. [CrossRef]

52. Centeno-Betanzos, L.Y.; López-Caamal, A.; Cortés Rendon, N.; León Santiago, M.; Osorio, E.; Bastida-Armengol, J.; Cano-Santana, Z.; Reyes-Chilpa, R.; Tovar-Sánchez, E. Microsatellites, morphological, and alkaloids characterization of *Zephyranthes fosteri* and *Z. alba* (Amaryllidaceae): Allopatric populations. *Biochem. Syst. Ecol.* **2022**, *101*, 104398. [CrossRef]
53. Ocampo-Bautista, F.; Mussali-Galante, P.; Alvarez, L.; Marquina-Bahena, S.; Valencia-Cuevas, L.; Valencia, A.S.; Tovar-Sánchez, E. Natural Hybridization between *Bursera bicolor* × *B. glabrifolia* (Burseraceae) Complex: Molecular and Chemical Evidence. *Forests* **2023**, *14*, 1382. [CrossRef]
54. López-Caamal, A.; Mussali-Galante, P.; Valencia-Cuevas, L.; JiménezRamírez, J.; Vega Flores, K.; Tovar-Sánchez, E. Transgressive character expression in hybrid zones between the native invasives *Tithonia tubaeformis* and *Tithonia rotundifolia* (Asteraceae) in Mexico. *Plants Syst. Evol.* **2013**, *299*, 1781–1792. [CrossRef]
55. Ferrusquía-Villafranca, I. Geology of Mexico: A synopsis. In *Biological Diversity of Mexico: Origins and Distribution*; Ramamoorthy, T.P., Bye, R., Lot, A., Fa, J., Eds.; Oxford University Press: New York, NY, USA, 1993; pp. 3–107.
56. Suárez-Mota, M.E.; Téllez-Valdés, O.; Martínez-Meyer, E. Dominios climáticos de las áreas naturales protegidas del eje volcánico transversal de México. *GeoFocus* **2014**, *14*, 120–143.
57. Maya, J.O.M.; López, Á.L. *Geografía de México. Una Reflexión Espacial Contemporánea*; Universidad Nacional Autónoma de México, Instituto de Geografía: Mexico City, Mexico, 2016.
58. Steinkellner, H.; Lexer, C.; Turetschek, E.; Gl€ossl, J. Conservation of $(GA)_n$ microsatellite loci between *Quercus* species. *Mol. Ecol.* **1997**, *6*, 1189–1194. [CrossRef]
59. Kampfer, S.; Lexer, C.; Gl€ossl, J.; Steinkellner, H. Brief report characterization of $(Ga)_n$ microsatellite loci from *Quercus robur*. *Heredita* **1998**, *129*, 183–186. [CrossRef]
60. Aldrich, P.R.; Michler, C.H.; Sun, W.; Romero-Severson, J. Microsatellite markers for northern red oak (Fagaceae: *Quercus rubra*). *Mol. Ecol. Notes* **2002**, *2*, 472–474. [CrossRef]
61. Sánchez-Chardi, A.; Nadal, J. Bioaccumulation of metals and effects of landfill pollution in small mammals. Part I. The greater white-toothed shrew, *Crocidura russula*. *Chemosphere* **2007**, *68*, 703–711. [CrossRef] [PubMed]
62. Weld, L.H. *Cynipoidea (Hym.) 1905–1950 Being a Supplement to the Dalla Torre and Kieffer Monograph the Cynipidae in Das Tierreich, Leiferung 24, 1910 and Bringing the Systematic Literature of the World up to Date, Including Keys to Families and Subfamilies and List of New Generic, Specific and Variety Names*; Privately printed: Ann Arbor, MI, USA, 1952; 351p.
63. Kinsey, A.C. The Origin of Higher Categories in Cynips. *Indiana Univ. Publ. Sci. Ser.* **1936**, *4*, 1–334.
64. Kinsey, A.C. New Mexican Gall Wasps (Hymenopter, Cynipidae) IV. *Proc. Indian Acad. Sci.* **1937**, *47*, 261–280.
65. Anantanarayanan, R.; Schaefer, C.W.; Wither, T.M.; Raman, A.; Schaefer, C.W.; Withers, T.W. *Biology, Ecology and Evolution of Gall-Inducing Arthropods*; Science Publishers, Inc.: Plymouth, UK, 2005; pp. 573–642.
66. Melika, G.; Cibrián-Tovar, D.; Cibrián-Llanderal, V.D.; Tormos, J.; Pujade-Villar, J. New species of oak gallwasp from Mexico (Hymenoptera: Cynipidae: Cynipini) a serious pest of *Quercus laurina* (Fagaceae). *Dugesiana* **2009**, *16*, 67–73.
67. Pujade-Villar, J.; Romero-Rangel, S.; Chagoyán-García, C.; Equihua-Martínez, A.; Estrada-Venegas, E.G.; Melika, G. A new genus of oak gallwasps, Kinseyella Pujade-Villar & Melika, with a description of a new species from Mexico (Hymenoptera: Cynipidae: Cynipini). *Zootaxa* **2010**, *2335*, 16–28. [CrossRef]
68. Melika, G.; Equihua-Martínez, A.; Estrada-Venegas, E.G.; Cibrián-Tovar, D.; Cibrián-Llanderal, V.D.; Pujade-Villar, J. New *Amphibolips* gallwasp species from Mexico (Hymenoptera: Cynipidae). *Zootaxa* **2011**, *3105*, 47–59. [CrossRef]
69. Nieves-Aldrey, J.L.; Pascual, E.; Maldonado-López, Y.; Medianero, E.; Oyama, K. Revision of the *Amphibolips* species of Mexico excluding the "niger complex" Kinsey (Hymenoptera: Cynipidae), with description of seven new species. *Zootaxa* **2012**, *3545*, 1–40. [CrossRef]
70. Pujade-Villar, J.; Equihua-Martínez, A.; Estrada-Venegas, E.G.; Lomelí-Flores, J.R.; Serrano-Muñoz, M.; Cabral, O.; Treto, R.; Landa, L.; Carrillo, C.; Cibrián-Tovar, D.; et al. Aportaciones de 2010–2011 en el conocimiento de los Cynipidae Mexicanos (Hym. Cynipidae, Cynipini) y perspectivas. *Entomol. Mex.* **2012**, *11*, 1057–1062.
71. Pujade-Villar, J.; Ferrer-Suay, M. Adjudicació genérica d' espècies mexicanes d'ubicació dudtosa descrites per Kinsey i comentaris sobre la fauna mexicana (Hymenoptera: Cynipidae: Cynipini). *Bull. Inst. Catalana Hist. Nat.* **2015**, *79*, 7–14.
72. Pujade-Villar, J.; Jiménez-Quiróz, E.; Trejo-Ramirez, O.; Antonio-Olivo, J.; Ferrer-Suay, M. Una especie de avispa gallícola introducida en el estado de Chihuahua procedente de Estados Unidos: *Andricus quercuslanigera* (Ashmead, 1881) (Hymenoptera: Cynipidae). *Entomol. Mex.* **2016**, *3*, 602–608.
73. Pujade-Villar, J.; Serrano-Muñoz, M.; García-Martiñón, R.D.; Villegas-Guzmán, G.A.; Equihua-Martínez, A.; Estrada-Venegas, E.G.; Ferrer-Suay, M. Una especie nueva de avispa gallícola para México; *Andricus sphaericus* Pujade-Villar n. sp. (Hymenptera: Cynipidae: Cynipini). *Dugesiana* **2016**, *23*, 15–20. [CrossRef]
74. Martínez-Romero, A.; Cuesta-Porta, V.; Equihua-Martínez, A.; Estrada-Venegas, E.D.; Barrera-Ruiz, U.M.; Cibrián-Tovar, D.; Pujade-Villar, J. Contribution to the knowledge of the Cynipini species (Hymenoptera: Cynipidae) in the Mexican states. *Rev. Mex. Biodiver.* **2022**, *93*, e933998. [CrossRef]

75. Gibson, G.A.P.; Huber, J.T.; Woolley, J.B. *Annotated Keys to the Genera of Neartic Chalcidoidea (Hymenoptera)*; NRC Research Prees: Ottawa, ON, Canada, 1997.
76. Nieves-Aldrey, J.L. Hymenoptera, Cynipidae. In *Fauna Ibérica*; Ramos, M.A., Alba Tercedor, J., Bellés i Ros, X., Gosálbez i Noguera, J., Guerra Sierra, A., Macpherson Mayol, E., Martín Piera, F., Serrano Marino, J., Templado González, J., Eds.; Museo Nacional de Ciencias Naturales; CSIC: Madrid, Spain, 2001; Volume 16, 636p.
77. Gibson, G.A.P. Superfamilia Chalcidoidea. In *Introducción a Los Hymenoptera de la Región Neotropical*; Fernández, F., Sharkey, M.J., Eds.; Sociedad Colombiana de Entomología y Universidad Nacional de Colombia Bogotá D.C.: Bogotá, Colombia, 2006; pp. 629–645.
78. Gómez, J.F.; Nieves-Aldrey, J.L.; Hernández-Nieves, M.; Stone, G.N. Comparative morphology and biology of terminal-instar larvae of some *Eurytoma* (Hymenoptera, Eurytomidae) species parasitoids of gall wasps (Hymenoptera, Cynipidae) in western Europe. *Zoosystema* **2011**, *33*, 287–323. [CrossRef]
79. Tovar-Sánchez, E.; Valencia-Cuevas, L.; Mussali-Galante, P.; Ramírez-Rodríguez, R.; Castillo-Mendoza, E. Effect of host-plant genetic diversity on oak canopy arthropod community structure in central Mexico. *Rev. Chil. Hist. Nat.* **2015**, *88*, 12. [CrossRef]
80. Yeh, F.C.; Boyle, R.; Yang, R.C. POPGENE Version 1.32. Computer Program and Documentation Distributed by the Author. 1999. Available online: http://www.ualberta.ca/~fyeh/popgene.html (accessed on 31 July 2024).
81. Zar, J.H. *Biostatistical Analysis*; Prentice-Hall/Pearson: London, UK, 2010.
82. Solow, A.R. A simple test for change in community structure. *J. Anim. Ecol.* **1993**, *62*, 191–193. [CrossRef]
83. Statsoft Inc. *STATISTICA for Windows*; Statsoft Inc.: Tulsa, OK, USA, 2007.
84. Henderson, P.A.; Seaby, R.M. *Species Diversity and Richness, Version 3.02*; Pisces Conservation Ltd.: Lymington, UK, 2002.
85. Wehenkel, C.; Mariscal-Lucero, S.; Jaramillo-Correa, J.P.; López-Sánchez, C.A.; Vargas-Hernández, J.J.; Sáenz-Romero, C. Genetic diversity and conservation of Mexican forest trees. In *Biodiversity and Conservation of Woody Plants*; Springer: Cham, Switzerland, 2016; pp. 37–67.
86. Oyama, K.; Ramírez-Toro, W.; Peñaloza-Ramírez, J.M.; Pérez-Pedraza, A.E.; Torres-Miranda, C.A.; Ruiz-Sánchez, E.; González-Rodríguez, A. High genetic diversity and connectivity among populations of *Quercus candicans*, *Quercus crassifolia*, and *Quercus castanea* in a heterogeneous landscape in Mexico. *Trop. Conserv. Sci.* **2018**, *11*, 1–14. [CrossRef]
87. Pérez-Pedraza, A.; Rodríguez-Correa, H.; Valencia-Ávalos, S.; Torres-Miranda, C.A.; Arenas-Navarro, M.; Oyama, K. Effect of hybridization on the morphological differentiation of the red oaks *Quercus acutifolia* and *Quercus grahamii* (Fagaceae). *Plant Syst. Evol.* **2021**, *307*, 37. [CrossRef]
88. Tovar-Sánchez, E.; Mussali-Galante, P.; Esteban-Jiménez, R.; Piñero, D.; Arias, D.M.; Dorado, O.; Oyama, K. Chloroplast DNA polymorphism reveals geographic structure and introgression in the *Quercus crassipes* × *Quercus crassifolia* hybrid complex in Mexico. *Botany* **2008**, *86*, 228–239. [CrossRef]
89. Madritch, M.D.; Hunter, M.D. Phenotypic diversity influences ecosystem functioning in an oak sandhills community. *Ecology* **2002**, *83*, 2084–2090. [CrossRef]
90. Lynch, M.; Walsh, B. *Genetics and Analysis of Quantitative Traits*; Sinauer: Sunderland, MA, USA, 1998.
91. Kai, K.; Shimizu, B.; Mizutani, M.; Watanabe, K.; Sakata, K. Accumulation of coumarins in *Arabidopsis thaliana*. *Phytochemistry* **2006**, *67*, 379–386. [CrossRef]
92. Tsai, H.H.; Schmidt, W. Mobilization of iron by plant-borne coumarins. *Trends Plant Sci.* **2017**, *22*, 538–548. [CrossRef] [PubMed]
93. Barker, H.L.; Holeski, L.M.; Lindroth, R.L. Genotypic variation in plant traits shapes herbivorous insect and ant communities on a foundation tree species. *PLoS ONE* **2018**, *13*, e0200954. [CrossRef]
94. Klaper, R.; Ritland, K.; Mousseau, T.A.; Hunter, M.D. Heritability of phenolics in *Quercus laevis* inferred using molecular markers. *J. Hered.* **2001**, *92*, 421–426. [CrossRef] [PubMed]
95. Rehill, B.J.; Whitham, T.G.; Martinsen, G.D.; Schweitzer, J.A.; Bailey, J.K.; Lindroth, R.L. Developmental trajectories in cottonwood phytochemistry. *J. Chem. Ecol.* **2006**, *32*, 2269–2282. [CrossRef] [PubMed]
96. Hartley, S.E.; Schen, R.; Hordwood, J.M.; Robinson, L.; Hill, E.M. Plant secondary metabolites and the interactions between plants and other organisms: The potential of a metabolomic approach. In *The Ecology of Plant Secondary Metabolites: From Genes to Global Processes*; Cambridge University Press: New York, NY, USA, 2012; pp. 191–203.
97. Crawford, D.J. A morphological and chemical study of *Populus acuminata* Rydberg. *Brittonia* **1974**, *26*, 74–89. [CrossRef]
98. Cheynier, V.; Comte, G.; Davies, K.M.; Lattanzio, V.; Martens, S. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiol. Biochem.* **2013**, *72*, 1–20. [CrossRef] [PubMed]
99. de Beaulieu, H.A.; Lamant, T. *Guide Illustré Des Chenes*, 2nd ed.; Tome 1; Edilens: Geer, Belgique, 2010.
100. García-Martiñón, R.D.; Equihua-Martínez, A.; Estrada-Venegas, E.G.; Acuña-Soto, J.A.; Pujade-Villar, J. Cynipidae asociados a encinos (Hym., Cynipidae: Cynipini) en los municipios de san Felipe del progreso y Jocotitlán (Estado de México). *Entomol. Mex.* **2018**, *5*, 444–452.

101. Pascual-Alvarado, E.; Nieves-Aldrey, J.L.; Castillejos-Lemus, D.E.; Cuevas-Reyes, P.; Oyama, K. Diversity of galls induced by wasps (Hymenoptera: Cynipidae, Cynipini) associated with oaks (Fagaceae: *Quercus*) in Mexico. *Bot. Sci.* **2017**, *95*, 461–472. [CrossRef]
102. Serrano-Muñoz, M. Diversidad de Cinípinos (Hymenoptera: Cynipidae) y de Himenópteros (Synergini y Chalcidoidea) Asociados a Agallas de Encinos de la Región Noroeste de la Sierra de Guadalupe. Master's Thesis, Instituto Politécnico Nacional, Mexico City, Mexico, 2016.
103. Stone, G.N.; Schönrogge, K. The adaptive significance of insect gall morphology. *Trends Ecol. Evol.* **2003**, *18*, 512–522. [CrossRef]
104. Bailey, R.; Schönrogge, K.; Cook, J.M.; Melika, G.; Csóka, G.; Thuróczy, C.; Stone, G.N. Host niches and defensive extended phenotypes structure parasitoid wasp communities. *PLoS Biol.* **2009**, *7*, e1000179. [CrossRef]
105. Seigler, D.S. Shikimic acid pathway. In *Plant Secondary Metabolism*; Springer: Boston, MA, USA, 1998; pp. 94–105.
106. Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angew Chem. Int.* **2011**, *50*, 586–621. [CrossRef] [PubMed]
107. Dungey, H.S.; Potts, B.M.; Whitham, T.G.; Li, H.F. Plant genetics affects arthropod community richness and composition: Evidence from a synthetic eucalypt hybrid population. *Evolution* **2000**, *54*, 1938–1946. [CrossRef]
108. Hochwender, C.G.; Fritz, R.S. Plant genetic differences influence herbivore community structure: Evidence from a hybrid willow system. *Oecologia* **2004**, *138*, 547–557. [CrossRef]
109. Bailey, J.K.; Schweitzer, J.A.; Rehill, B.; Lindroth, R.; Whitham, T.G. Beavers as molecular geneticists: A genetic basis to the foraging of an ecosystem engineer. *Ecology* **2004**, *85*, 603–608. [CrossRef]
110. Bailey, J.K.; Whitham, T.G. Interactions between cotton wood and beavers positively affect sawfly abundance. *Ecol. Entomol.* **2006**, *31*, 294–297. [CrossRef]
111. Fritz, R.S.; Hochwender, C.G.; Brunsfeld, S.J.; Roche, B.M. Genetic architecture of susceptibility to herbivores in hybrid willows. *J. Evol. Biol.* **2003**, *16*, 1115–1126. [CrossRef] [PubMed]
112. Whitlock, R. Relationships between adaptive and neutral genetic diversity and ecological structure and functioning: A meta-analysis. *J. Ecol.* **2014**, *102*, 857–872. [CrossRef] [PubMed]
113. Bailey, J.K.; Wooley, S.C.; Lindroth, R.L.; Whitham, T.G. Importance of species interactions to community heritability: A genetic basis titrophic-level interaction. *Ecol. Lett.* **2006**, *9*, 78–85. [CrossRef]
114. Johnson, M.T.J. Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology* **2008**, *89*, 145–154. [CrossRef] [PubMed]
115. Bidart-Bouzat, M.G.; Kliebenstein, D.J. Differential levels of insect herbivory in the field associated with genotypic variation in glucosinolates in *Arabidopsis thaliana*. *J. Chem. Ecol.* **2008**, *34*, 1026–1037. [CrossRef] [PubMed]
116. Koricheva, J.; Hayes, D. The relative importance of plant intraspecific diversity in structuring arthropod communities: A meta-analysis. *Funct. Ecol.* **2018**, *32*, 1704–1717. [CrossRef]
117. Pagare, S.; Bhatia, M.; Tripathi, N.; Pagare, S.; Bansal, Y.K. Secondary metabolites of plants and their role: Overview. *Curr. Trends Biotechnol. Pharm.* **2015**, *9*, 293–304.
118. Stahl, E.; Hilfiker, O.; Reymond, P. Plant–arthropod interactions: Who is the winner? *Plant J.* **2018**, *93*, 703–728. [CrossRef]
119. Tooker, J.F.; Hauser, M.; Hanks, L.M. Floral host plants of Syrphidae and Tachinidae (Diptera) of central Illinois. *Ann. Entomol. Soc. Am.* **2006**, *99*, 96–112. [CrossRef]
120. Hall, A.A.G.; Johnson, S.N.; Cook, J.M.; Riegler, M. High nymphal host density and mortality negatively impact parasitoid complex during an insect herbivore outbreak. *Insect Sci.* **2019**, *26*, 351–365. [CrossRef]
121. Graziosi, I.; Rieske, L.K. Response of *Torymus sinensis*, a parasitoid of the gall forming *Dryocosmus kuriphilus*, to olfactory and visual cues. *Biol. Control* **2013**, *67*, 137–142. [CrossRef]
122. Borges, R.M. The galling truth: Limited knowledge of gall-associated volatiles in multitrophic interactions. *Front. Plant Sci.* **2018**, *9*, 1139. [CrossRef]
123. Steck, W. The biosynthetic pathway from caffeic acid to scopoline in tobacco leaves. *Can. J. Biochem.* **1967**, *45*, 1995–2003. [CrossRef] [PubMed]
124. Gnonlonfin, G.J.B.; Sanni, A.; Brimer, L. Review scopoletin—A coumarin phytoalexin with medicinal properties. *Crit. Rev. Plant Sci.* **2012**, *31*, 47–56. [CrossRef]
125. Murray, R.D.H.; Mendez, J.; Brown, S.A. *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*; Wiley: New York, NY, USA, 1982.
126. Sun, H.; Wang, L.; Zhang, B.; Ma, J.; Hettenhausen, C.; Cao, G.; Sun, G.; Wu, J.; Wu, J. Scopoletin is a phytoalexin against *Alternaria alternata* in wild tobacco dependent on jasmonate signalling. *J. Exp. Bot.* **2014**, *65*, 4305–4315. [CrossRef] [PubMed]
127. Taper, M.L.; Zimmerman, E.M.; Case, T.J. Sources of mortality for a cynipid gall-wasp (*Dryocosmus dubiosus* (Hymenoptera: Cynipidae)): The importance of the Tannin/Fungus interaction. *Oecologia* **1986**, *68*, 437–445. [CrossRef] [PubMed]
128. Simmonds, M.S.J. The search for plant-derived compounds with antifeedant activity. In *Naturally Occurring Bioactive Compounds*; Elsevier: Amsterdam, The Netherlands, 2006; pp. 291–323.

129. Mori, M. n-hexacosanol and n-octacosanol: Feeding stimulants for larvae of the silkworm, *Bombyx mori*. *J. Insect Physiol.* **1982**, *28*, 969–973. [CrossRef]
130. Lin, S.; Mullin, C.A. Lipid, polyamide, and flavonol phagostimulants for adult western corn rootworm from sunflower (*Helianthus annuus* L.). *Pollen. J. Agric. Food Chem.* **1999**, *47*, 1223–1229. [CrossRef] [PubMed]
131. Bruce, T.J.A. Glucosinolates in oilseed rape: Secondary metabolites that influence interactions with herbivores and their natural enemies. *Ann. Appl. Biol.* **2014**, *164*, 348–353. [CrossRef]
132. Nebapure, S.M.; Sagar, D. Insect-plant interaction: A road map from knowledge to novel technology. *Karnataka J. Agric. Sci.* **2015**, *28*, 1–7.
133. Simmonds, M.S. J Importance of flavonoids in insect-plant interactions: Feeding and oviposition. *Phytochemistry* **2001**, *56*, 245–252. [CrossRef]
134. Mierziak, J.; Kostyn, K.; Kulma, A. Flavonoids as important molecules of plant interactions with the environment. *Molecules* **2014**, *19*, 16240–16265. [CrossRef]
135. Tavares, W.S.; Pereira, A.I.A.; Freitas, S.S.; Serrão, J.E.; Zanuncio, J.C. The chemical exploration of *Dimorphandra mollis* (Fabaceae) in Brazil, with emphasis on insecticidal response: A review. *J. Sci. Ind. Res.* **2014**, *73*, 465–468.
136. Meyuhas, S.; Assali, M.; Huleihil, M.; Huleihel, M. Antimicrobial activities of caffeic acid phenethyl ester. *J. Mol. Biochem.* **2015**, *4*, 21–31.
137. Nakhaie-Bahrami, M.; Mikani, A.; Moharramipour, S. Effect of caffeic acid on feeding, α-amylase and protease activities and allatostatin-A content of Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J. Pestic. Sci.* **2018**, *43*, 73–78. [CrossRef] [PubMed]
138. Xiao-Ju, Y.; Yongqiang, Z.; Wei, D. Sublethal effects of scopoletin on the experimental population of the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae). *Acta Entomol. Sin.* **2018**, *54*, 1377–1383.